

1 **SUPPLEMENTARY FIGURE LEGENDS**

2

3 **Figure S1. Identification of the Ser⁶⁵ phosphorylation site by Edman**
4 **sequencing and mass spectrometry.** Phosphopeptides P2 (A) and P1 (B) from
5 Figure 1C were sequenced by solid-phase Edman degradation using an Applied
6 Biosystems 494C sequencer after the peptides were coupled to Sequelon-arylamine
7 membrane (Applied Biosystems) as described previously (Campbell and Morrice
8 2002). The amino acid sequence deduced from the LC-MS-MS analysis is shown
9 using the single-letter code for amino acids.

10

11 **Figure S2. Mapping of PINK1 cleavage site by N-terminal Edman sequencing.**
12 HEK293 cells were transiently transfected with wild-type PINK1-FLAG and 100 mg
13 of whole cell lysate immunoprecipitated with anti-FLAG agarose. After
14 electrophoresis, samples were transferred to Immobilon PVDF membrane and
15 stained with Coomassie Blue. (A) Coomassie stained PVDF membrane showing band
16 corresponding to the cleaved form of PINK1 that was excised and subjected to
17 Edman degradation and analysis. The amino acid sequence obtained in the gel band
18 started with FGLGLG (residues 104 – 109). Representative of 3 independent
19 experiments. (B) Sequence alignment of residues around Phe¹⁰⁴ in human PINK1
20 showing high degree of conservation amongst higher organisms. Cleavage site
21 indicated by an arrow.

22

23 **Figure S3. Mass spectrometry confirmation that phosphorylation of PINK1**
24 **Thr²⁵⁷ is an autophosphorylation site.**

25 Flp-In T-Rex HEK 293 cell line stably expressing wild-type or kinase-inactive PINK1-
26 FLAG (D384A) were treated 10 µM of CCCP for 3 h. (A) Recombinant PINK1 was
27 immunoprecipitated from 10mg of mitochondrial extract for each condition using
28 anti-FLAG-agarose, subjected to 4-12% gradient SDS-PAGE, and stained with
29 colloidal Coomassie blue. (B) The Coomassie-stained bands migrating with the
30 expected molecular mass of PINK1-FLAG were excised from the gel, digested with
31 trypsin, and subjected to LC-MS-MS on an LTQ-Orbitrap mass spectrometer. The
32 Thr²⁵⁷ phosphopeptide was only detected in the wild-type PINK1-FLAG band.

33

34 **Figure S4. Structure of human Parkin Ubl domain.** Three views representing a
35 90° rotation about the y-axis, depict the Ubl domain of Parkin with Ser⁶⁵ highlighted
36 and the contacts it make within 4 Angstroms (PDB code 1IYF).

37

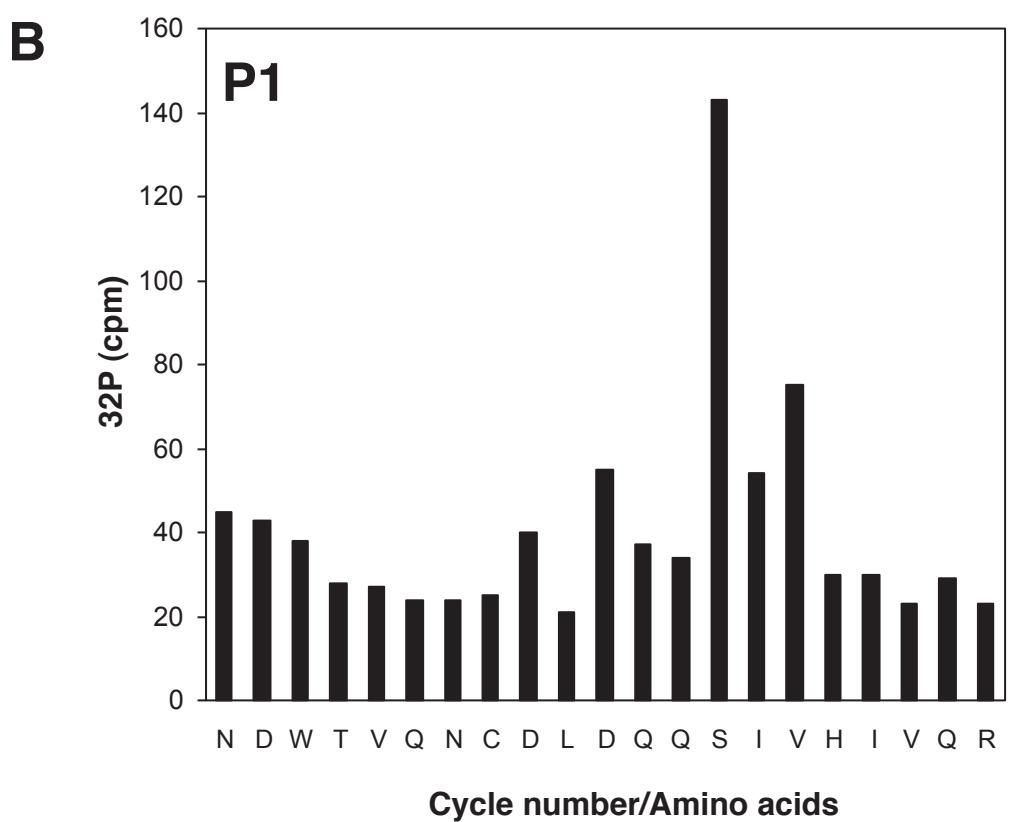
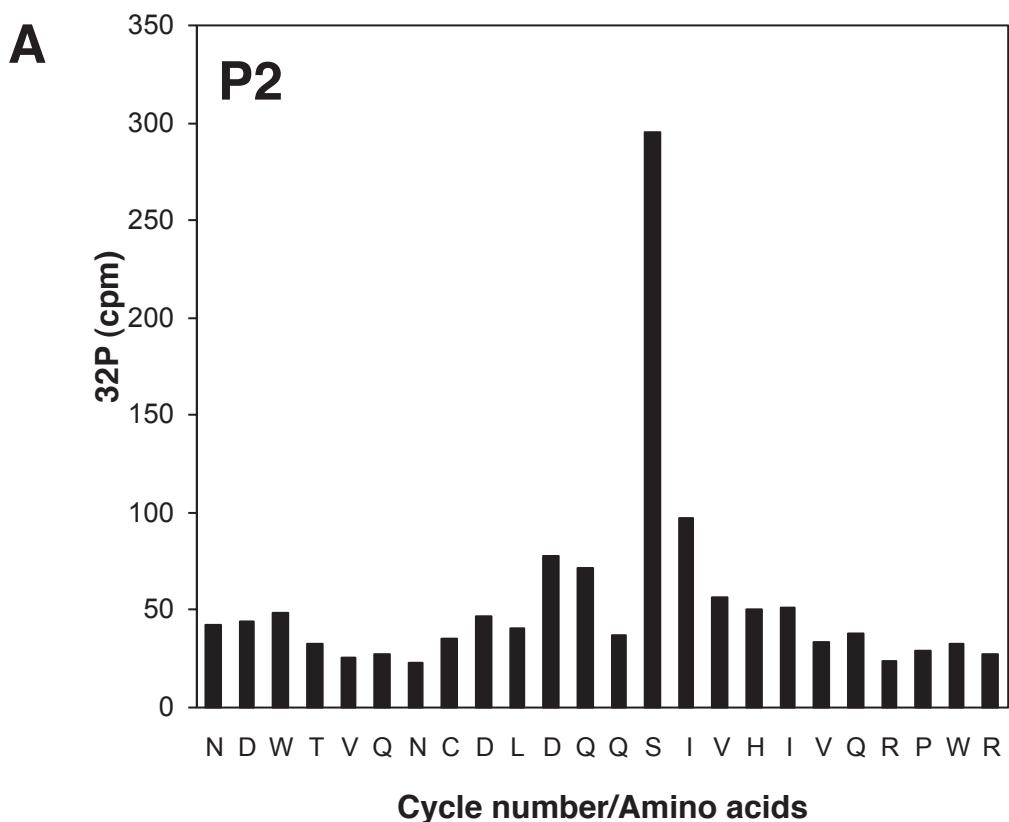
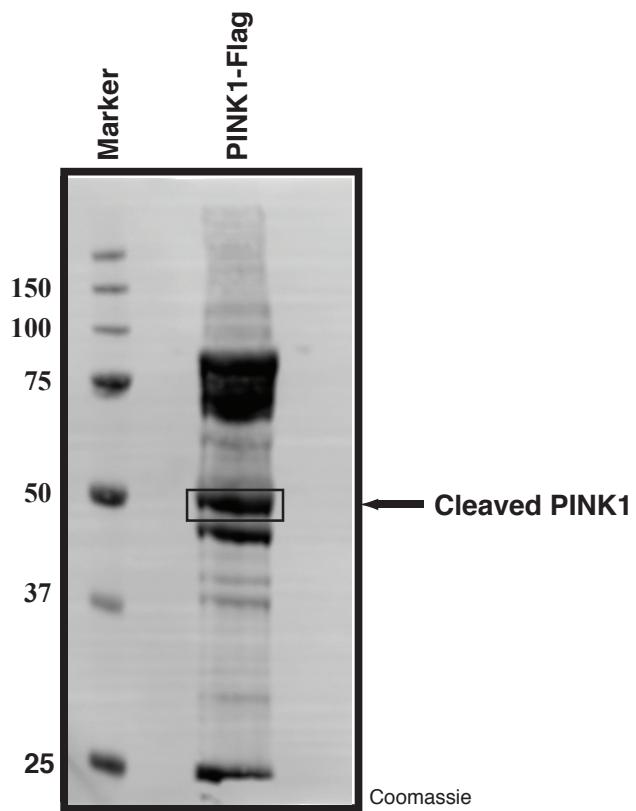


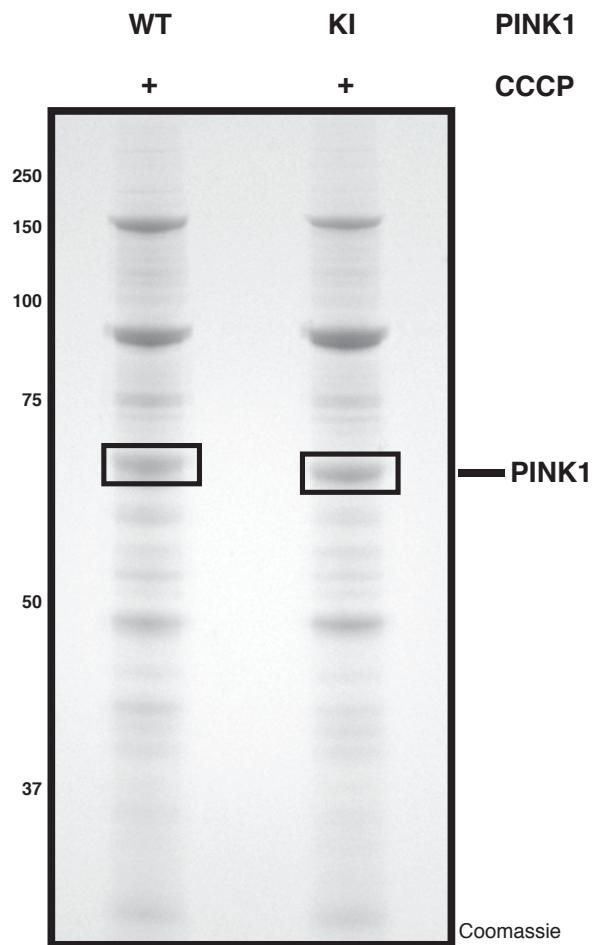
Figure S1



Cleavage site between residues 103-104

<i>H. sapiens</i>	98	RAVFLA-FGLGLGLI
<i>M. musculus</i>	98	RAVFLA-FGLGLGLI
<i>R. norvegicus</i>	98	RAVFLA-FGLGLGLI
<i>B. taurus</i>	101	RAVFLA-FGLGLGLI
<i>D. rerio</i>	88	RAVFLA-FGVGLGLI
<i>M. domestica</i>	98	RAVFLA-FGLGLGLI
<i>M. fascicularis</i>	58	RAVFLA-FGLGLGLI
<i>M. mulatta</i>	98	RIVFLA-FGLGLGLI
<i>P. abelii</i>	98	RAVFLA-FGLGLGLI

Figure S2

A**B**

Observed Mass (m/z)	Theoretical Mass (m)	Sequence	Phosphorylated Residue	No. of experiments detected
789.38	1576.76	VALAGEYGAV <p>T</p> YRK	257	2

Figure S3

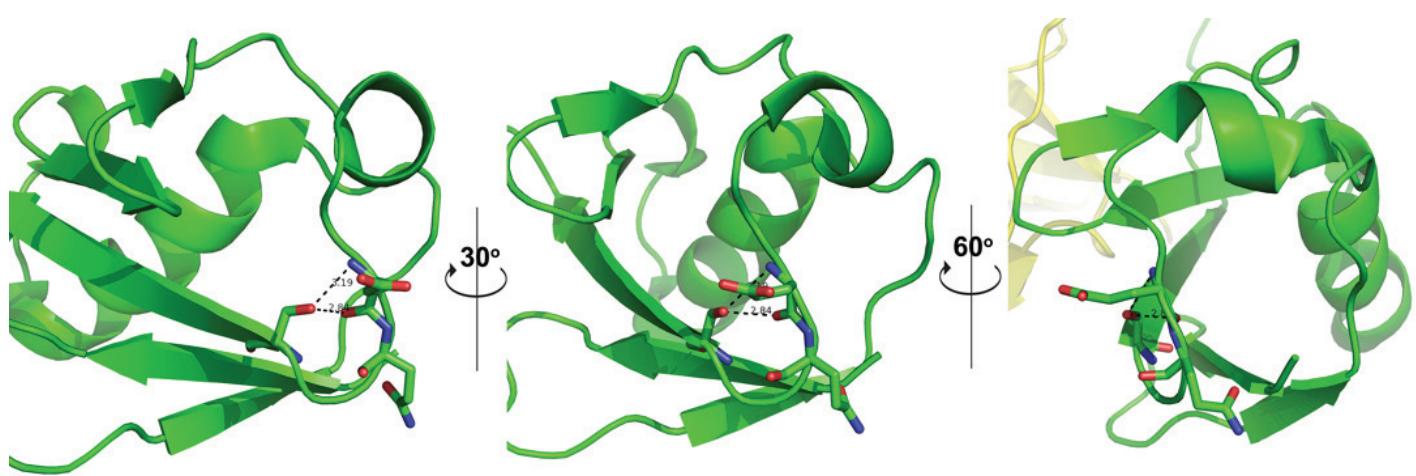


Figure S4